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Biochemical evaluation of hypothyroidism and euthyroid state in cases of pregnancy by the estimation of T3, T4 and TSH

K. Maria Kumar[1*], Vijaya Kumari[2], P. Sateesh[3]

[1]Associate Professor, [2]Professor & HOD, [3]Postgraduate 2nd Year, Dept of Biochemisty, Guntur Medical College, Andhra Pradesh, India.

***Corresponding Author: K. Maria Kumar**
Email: mariyakumar7kakumanu@gmail.com

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Abstract

Introduction: Thyroid diseases are not uncommon in women of reproductive age with an estimation of 5-15%. The thyroid gland must produce 50% more thyroid hormone in pregnancy in order to maintain euthyroidism and for foetal wellbeing.

Aims and Objectives: To study the effect of hypothyroidism in pregnant women, attending for ante-natal check-up to the dept. of OBG – GGH, Guntur.

Inclusion Criteria: Pregnant women with hypothyroidism with or without treatment, pregnant woman in euthyroid state.

Exclusion Criteria: Pregnant women with hyperthyroidism, smoking alcoholism, chronic infections, systemic illness and diabetes/impaired glucose tolerance and hypertension.

Materials and Methods: Singleton pregnant women aged ≥ 18 years TSH >2.5μIU/ml in first trimester and TSH >3μIU/ml in second and third trimester with normal T4 levels are included as cases. Controls are age and trimester matched healthy first, second and third trimester, singleton euthyroid pregnant women.

Results: The present study was carried out on age matched 120 pregnant women of which 60 are hypothyroid and 60 are euthyroid. Out of 60 cases 3 cases (5%) are with overt hypothyroidism and 57 cases (95%) are with subclinical hypothyroidism.

Keywords: Overt thyroid, Subclinical thyroid, TSH-Thyroid stimulating hormone, Chemiluminescence, Maternal serum sample.

Introduction

Thyroid diseases are not uncommon in women of reproductive age with an estimation of 5-15%. The thyroid gland must produce 50% more thyroid hormone in pregnancy in order to maintain euthyroidism and for foetal well-being. The physiological changes that accompany pregnancy results in marked alteration in the normal range of thyroid function. Specifically human chorionic gonadotropin which peaks in the first trimester cross reacts with the thyrotropin(TSH] receptors resulting in an upper limit of normal of thyrotropin of 2.5μ I.U /ml during the first trimester.[1-4] Oestrogen mediation(word mediation deleted) causes increase in maternal TBG. It increases primarily because of decreased clearance.[5] Enhanced hepatic synthesis and reduced degradation rate because of oligosaccharide modification.[6] As per ATA 2017 guidelines, in pregnancy subclinical hypothyroidism is defined as serum TSH between 2.5 and 10μIU/ml with a normal free or total thyroxin concentration, TSH 10IU/ml and normal free or total thyroxin considered as overt hypothyroidism. TSH reference range should be 0.1-2.5 μIU/ml during first trimester and 0.2-3.0μ IU/ml in the second and third trimester.[7,8] The pathologically enlarged thyroid gland depends on the aetiology of hypothyroidism and is more likely in women in areas of endemic iodine deficiency (more in developing countries) or those with hashimotos thyroiditis (more in developed countries). Other clinical findings include oedema, dry skin, hair loss and prolonged relaxation phase of deep tendon reflex.[9] Subclinical hypothyroidism is laboratory based biochemical diagnosis. In some studies women with subclinical hypothyroidism are at risk of adverse pregnancy complications such as miss carriage, preterm delivery, pre eclampsia, gestational hypertension, gestational diabetes and decreased I.Q, IUGR, low birth weight and cretinism in the offsprings.[10-13] The foetus is independent after 20wks of gestation. Various reports suggest that thyroid deficiency OH(overt hypothyroidism) and SCH (sub clinical hypothyroidism) during pregnancy results in impaired neuro development in off springs. Studies have documented the important role of T4 andT3 in neuronal growth, myelination, migration and organisation.[14-16]

Materials and Methods

This is an observational prospective study in the department of clinical Biochemistry, Govt.General hospital; Guntur. This present study included 120 pregnant women, of whom 60 are hypothyroid cases, and 60 are euthyroid controls. Singleton pregnant women aged 18 years under reproductive age group TSH >2.5μIU/ml in first trimester and TSH >3μIU/ml in second and third trimester with normal T4 levels are included as cases. Controls are age and trimester matched healthy first, second and third trimester, singleton euthyroid pregnant women.

The present study was carried out on age matched 120 pregnant women of which 60 are hypothyroid and 60 are euthyroid. Out of 60 cases 3 cases (5%) are with overt hypothyroidism and 57cases (95%) are with subclinical hypothyroidism based on TSH estimation. Out of 60 cases 34 (56.67%) has shown TSH range from 2.5-5.2μIU/ml (upper limit of reference range), and 23(38.33%) cases are with a range of 5.2 to10.0μIU/ml. more than 10μIU/ml was 3(5%) cases. Out of 60 cases raised levels of T3 was seen in 4(6.7%) cases.

Estimation of Total T3

Method: competitive binding immunoenzymatic assay
Instrument: BECKMAN COULTERACCESS2 chemiluminescence immune assay.

Principle: The Access total T3 assay is a competitive binding assay. Competitive binding assays use coated paramagnetic particles to measure antigen in a sample i.e T3 or T4. The particles can be either directly or indirectly coated with capture antibody. A sample is mixed with the paramagnetic particles, an antigen specific antibody and an enzyme-labelled analogue antigen(conjugate). The sample antigen competes with the conjugate for antibody binding sites. The antibody and conjugate form immune complexes that bind to the particles. Magnets separate the particle-bound immune complexes from the unbound components, and washingthree times removes the unbound components. After adding chemiluminescent substrate i.e dioxetane-P produces the light will be produced in terms of Relative Light Units (RLUs) which is inversely proportional to the amount of T3 in the sample. The amount of T3 in the sample is determined from a stored, multi-point calibration curve.

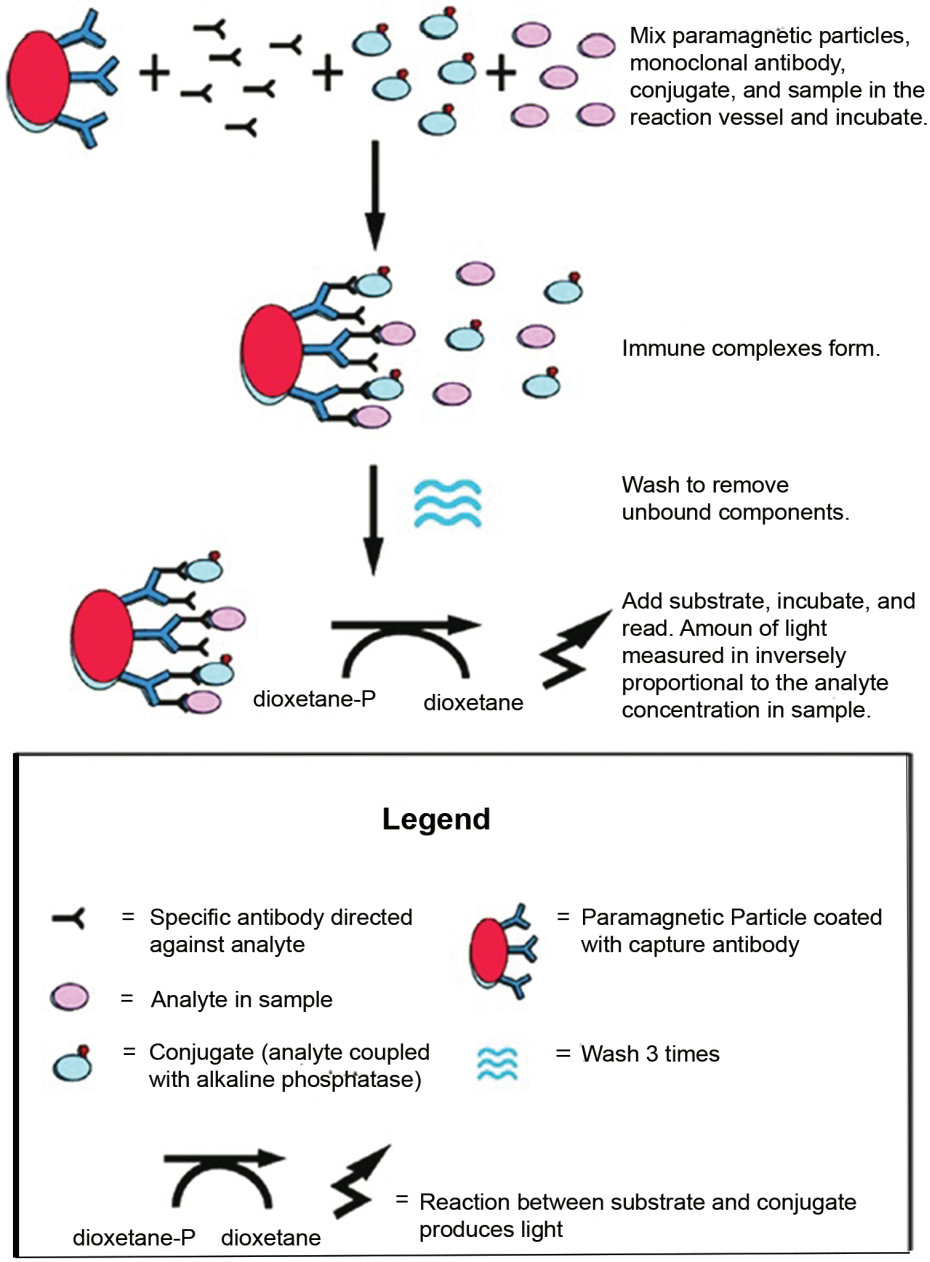
Calibrator: The Access Calibrators are provided at six levels S0 to S5 prepared gravimetrically from crystalline tri-iodothyronin and human sera.

S0, S1, S2, S3, S4, S5: Triiodothyronin(ng/ml) at levels of 0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 ng/ml

REFERENCE RANGE: 0.87 – 1.78ng/ml

Estimation of T4:

Method: competitive binding immunoenzymatic assay.
Instrument:BECKMAN COULTER ACCESS2 chemiluminescence immune assay.



Principle: the Access total T4 assay is a competitive binding immunoenzymatic assay as same as T3. After adding chemiluminescent substrate to the immune complexes of paramagnetic particles with capture antibodies and conjugate,T4 analogue coupled with alkaline phosphatase, specific antibody directed against T4 and T4 in the sample which is an antigen. This gives rise to light measured in terms of Relative Light Units which is inversely proportional to the T4 concentration in the sample.

Calibrator: The access Total T4 calibrators are provided at six levels S0 –S5 prepared gravimetrically from crystalline tetra-iodo thyronine and human sera.

S0, S1, S2, S3, S4, and **S5** contains 0.0µg/dl, 2.0, 4.0, 8.0, 16.0 and 30.0µg/dl

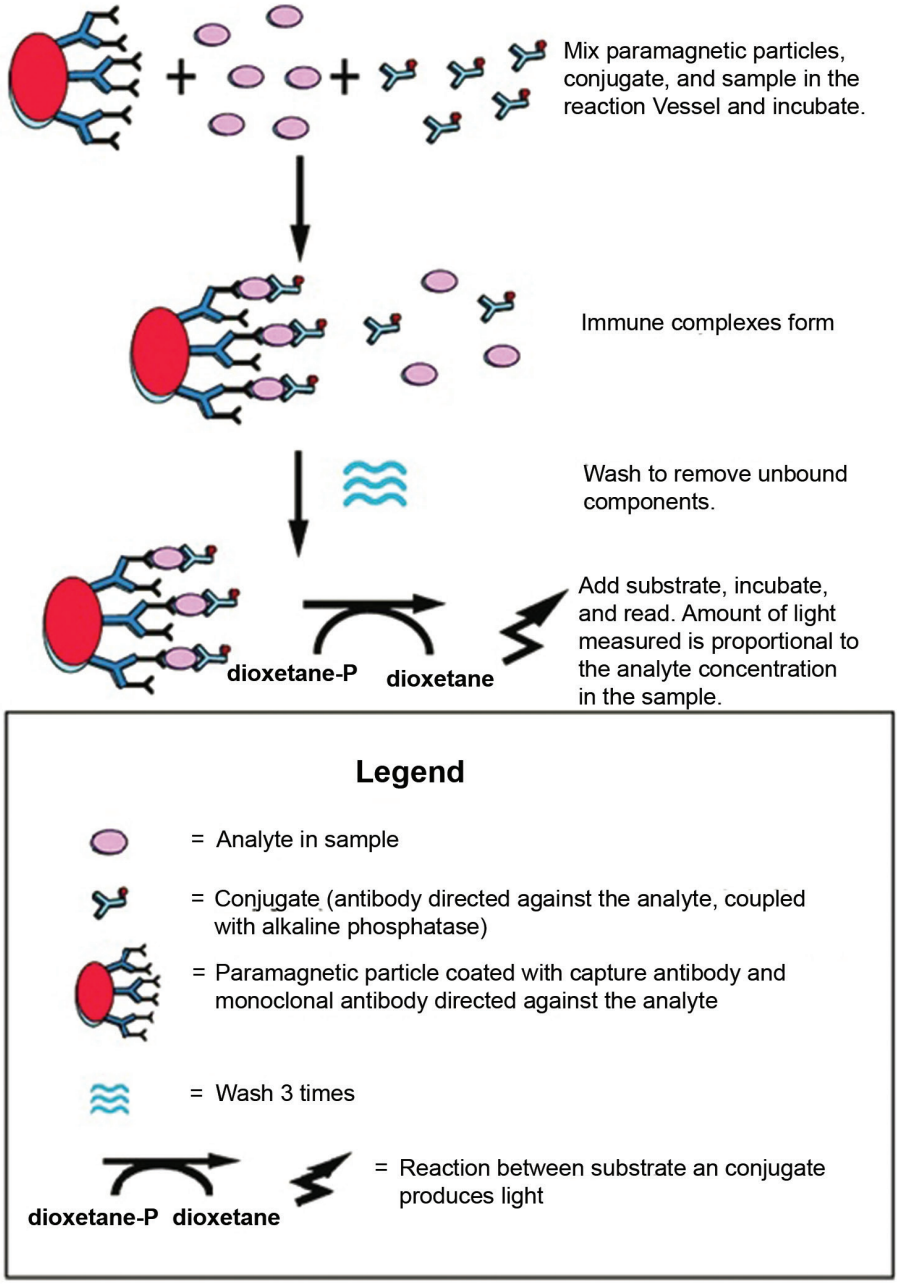
Reference Range: 4.82 to 15.65µg/dl

Estimation of TSH

Method: Two-site immunoenzymatic (sandwich) assay

Instrument: BECKMAN COULTER ACCESS2 chemiluminescence immune assay.

Principle: The Access hTSH (hypersensitive) (format changed) assay is a two site immunoenzymatic assay. It is also called as sandwich immunoassay use coated paramagnetic particles to measure antigen in the sample. The particles can be either directly or indirectly coated with capture antibody. A sample is mixed with the paramagnetic particles and an enzyme (alkaline phosphatase) labelled antibody (conjugate). The serum TSH and conjugate form immune complexes that bind to the paramagnetic particles with capture antibodies. Magnets separate the particle bound immune complexes from the unbound components and washing removes the unbound



components. After adding chemiluminescent substrate i.e. dioxetane-P the light will be generated which is measured in terms of Relative Light Units are directly proportional to the amount of TSH in the sample. The amount of TSH is determined from a stored multipoint calibration curve.

Calibrator: The access hTSH (hypersensitve) calibrators are provided at six levels prepared gravimetrically from human TSH and BSA matrix.

S0, S1, S2, S3, S4, S5 contain 0.1, 0.5, 4.0, 10.0 and 100.0 µIU/ml hTSH respectively.

Maternal serum samples are analysed for TSH, total T3, and total T4 by using chemiluminescence, access 2, Beckman Coulter.

The normal ranges of TSH, total T3, and total T4 are 0.1 to 2.5µIU/ml in first trimester and 0.2 to

3µIU/ml in second and third trimester.[8]

T3- 0.87 to 1.78ng/ml

T4- 4.82 to 15.65 µg/dl

For all the patients the following data is recorded

Blood samples are collected from both test and control groups for the analysis of following, by

using chemiluminescence immuno assay method.

1. Serum total T3 level(tri Iodothyronin)
2. Serum total T4(tetra Iodothyronin)
3. TSH-Thyroid stimulating hormone

Results

The present study was carried out on age matched 120 pregnant women of which 60 are hypothyroid and 60 are euthyroid. Out of 60 cases 3 cases (5%) are with overt hypothyroidism and 57cases (95%) are with subclinical hypothyroidism based on TSH estimation. Out of 60 cases 34 (56.67%) has shown TSH range from 2.5-5.2µIU/ml (upper limit of reference range), and 23(38.33%) cases are with a range of 5.2 to10.0µIU/ml. more than 10µIU/ml was 3(5%) cases. Out of 60 cases raised levels of T3 was seen in 4(6.7%) cases.

Table 1: Statistical analysis of T3

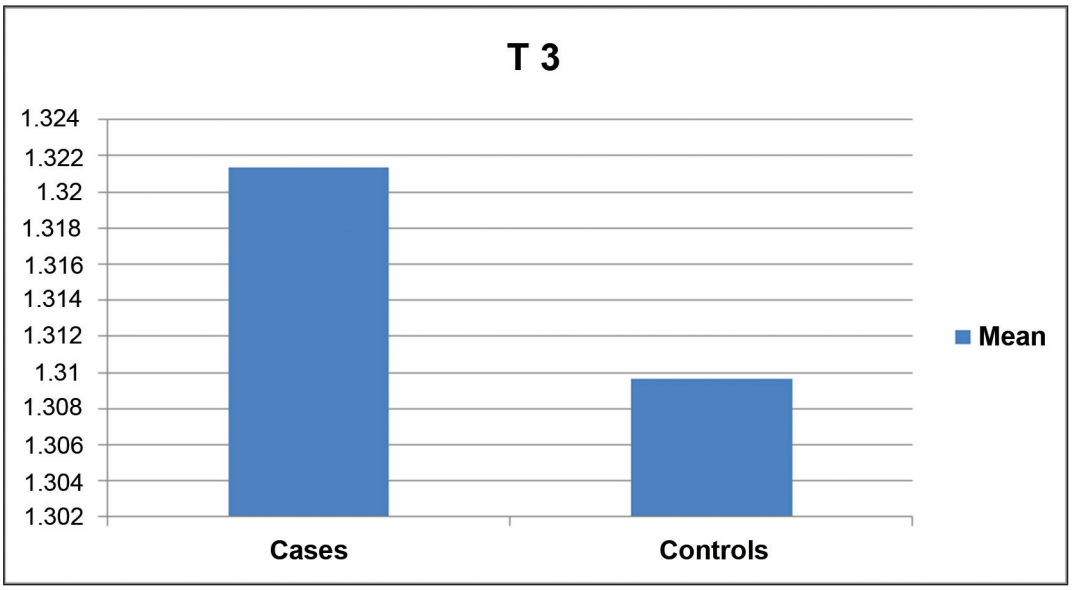


Table 2: Statistical analysis of T4

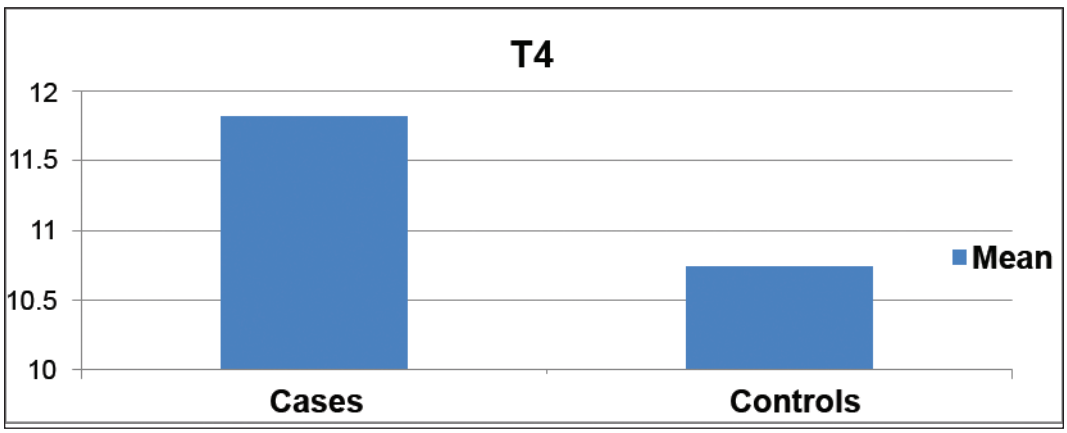


Table 3: Statistical analysis of TSH

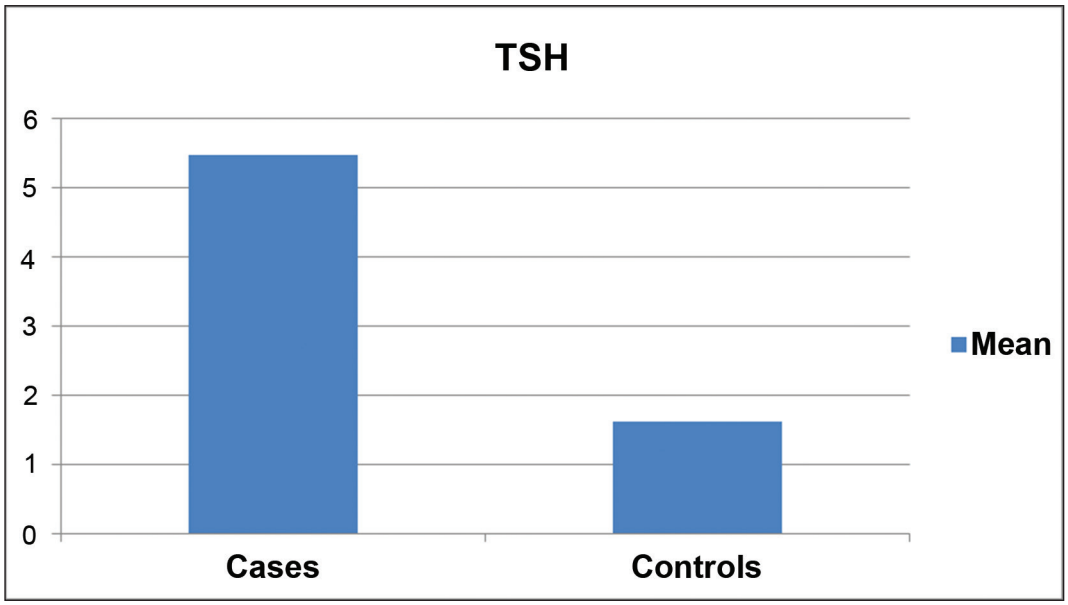


Table 4: Maser sheet for cases				
	Cases			
	Reference Units	**0.87-1.78 ng/mL**	**4.82-15.65(µg/dl)**	**0.34-5.6 µIU/ml**
S.No	**Age/sex**	**T3**	**T4**	**TSH**
1	25/F	1.60	9.65	6.63
2	23/F	1.98	12.25	7.0
3	22/F	0.94	8.19	4.67
4	19/F	1.16	12.19	3.50
5	20/F	1.18	10.50	3.28
6	25/F	1.48	10.52	4.40
7	22/F	1.44	13.25	6.18
8	20/F	1.28	10.97	5.65
9	23/F	1.49	11.38	3.24
10	20/F	1.40	11.05	3.05
11	26/F	1.72	12.60	3.02
12	21/F	1.36	10.85	3.84
13	26/F	1.02	10.91	5.63
14	20/F	0.97	11.16	4.06
15	25/F	1.37	12.35	4.42
16	21/F	1.22	12.09	3.69
17	34/F	1.22	10.37	4.47
18	35/F	1.07	8.88	6.02
19	22/F	1.48	16.95	3.72
20	27/F	1.43	11.25	3.63
21	28/F	1.55	10.94	3.19
22	22/F	1.76	11.31	4.60
23	23/F	1.52	8.80	4.16
24	24/F	1.03	10.00	3.44
25	28/F	1.39	9.08	3.67
26	27/F	0.93	8.87	4.50
27	21/F	1.13	12.69	5.15

28	25/F	1.40	10.64	5.99
29	20/F	1.06	10.15	4.31
30	23/F	1.50	12.12	4.04
31	25/F	1.40	6.55	7.00
32	25/F	1.36	9.94	5.78
33	18/F	1.02	10.18	5.37
34	24/F	1.64	18.63	6.13
35	23/F	1.98	12.25	7.0
36	20/F	1.38	8.97	9.28
37	20/F	1.19	9.97	9.84
38	23/F	1.43	13.92	8.61
39	22/F	1.00	10.23	9.0
40	23/F	1.48	13.53	5.05
41	20/F	1.67	17.73	3.03
42	20/F	1.35	14.75	4.23
43	20/F	1.18	17.00	6.43
44	22/F	1.44	13.25	6.18
45	19/F	1.24	14.56	6.24
46	30/F	1.26	11.03	4.19
47	21/F	1.55	16.00	5.25
48	19/F	1.54	18.14	3.48
49	20/F	1.20	11.91	3.40
50	27/F	1.06	13.79	5.28
51	24/F	1.08	13.87	5.37
52	23/F	1.29	14.27	7.14
53	20/F	1.62	14.39	5.00
54	21/F	1.50	14.54	5.14
55	19/F	1.66	15.12	5.80
56	22/F	1.13	12.10	7.60
57	21/F	1.20	12.39	4.06
58	19/F	0.17	0.80	10.30
59	20/F	0.96	10.48	10.94
60	28/F	1.22	7.05	13.56

Table 5: Master sheet for controls

	Controls			
	Reference Units	**0.87-1.78 ng/mL**	**4.82-15.65(µg/dl)**	**0.34-5.6 µIU/ml**
S.No	**Age/sex**	**T3**	**T4**	**TSH**
1	25/F	1.22	9.5	2.07
2	24/F	1.22	15.41	1.42
3	22/F	1.39	6.22	2.54
4	25/F	1.53	12.91	1.73
5	21/F	1.19	12.90	2.66
6	22/F	1.14	9.95	1.46
7	22/F	1.43	10.53	0.80
8	23/F	1.62	11.89	0.85
9	2O/F	1.18	13.41	0.43
10	20/F	1.45	7.16	2.43
11	21/F	1.55	14.33	1.09
12	26/F	1.33	12.28	2.48
13	22/F	1.27	12.58	1.04
14	22/F	1.32	13.81	1.47
15	25/F	2.12	14.42	0.54

(Continue...)

...(Continued) Table 5-master sheet for controls				
		Controls		
	Reference units	0.87-1.78 ng/mL	4.82-15.65(µg/dl)	0.34-5.6 µIU/ml
S.no	Age/sex	T3	T4	TSH
16	25/F	1.70	10.58	2.45
17	22/F	1.15	11.59	0.87
18	20/F	1.44	13.40	1.54
19	21/F	1.88	10.19	2.94
20	20/F	1.32	11.31	1.40
21	21/F	1.11	9.38	1.92
22	20/F	1.32	9.54	1.59
23	21/F	1.50	9.74	1.92
24	20/F	1.71	16.44	0.90
25	20/F	1.30	13	2.40
26	21/F	1.48	13.57	0.68
27	35/F	0.87	5.39	2.14
28	23/F	1.17	13.95	1.56
29	25/F	1.13	7.43	1.52
30	30/F	1.14	12.12	0.73
31	25/F	1.14	13.06	1.29
32	19/F	1.04	9.60	1.69
33	20/F	1.43	10.65	2.21
34	29/F	1.15	12.91	1.65
35	25/F	0.89	4.52	0.76
36	20/F	1.27	12.44	2.20
37	23/F	1.00	12.49	1.55
38	20/F	1.21	10.67	2.40
39	26/F	1.03	12.04	2.16
40	22/F	1.09	8.91	1.67
41	23/F	0.96	10.50	1.82
42	21/F	1.29	11.15	0.33
43	23/F	1.02	9.21	2.09
44	20/F	1.03	10.17	1.44
45	24/F	1.35	8.90	2.45
46	20/F	1.54	11.66	2.06
47	22/F	1.17	12.28	2.39
48	19/F	1.52	11.23	2.34
49	25/F	1.38	12.35	1.64
50	22/F	1.03	10.96	1.40
51	21/F	1.42	8.16	1.26
52	21/F	1.92	10.75	1.60
53	20/F	1.94	10.22	1.94
54	28/F	0.96	12.50	0.14
55	30/F	0.87	6.57	0.82
56	28/F	0.80	6.35	1.69
57	22/F	1.46	12.77	2.34
58	19/F	1.56	10.40	1.69
59	21/F	1.76	10.41	1.27
60	20/F	1.17	9.70	1.20

Results were collected, tabulated and statistically analysed. Microsoft XL was used for data entry and bar diagrams. The data was presented as mean and standard deviation (SD) for all three parameters. Two types of statistics were done. Descriptive statistics ex: AM and SD. Analytical statistics – P value was calculated.

P value<0.05 was considered statistically significant.
P value >0.05 was considered statistically non-significant.
P value <0.001was considered statistically highly significant.

The mean SD of T3 in hypothyroid cases are 1.32 0.28 on comparison with mean SD of controls are 1.30 0.28. P value is statistically non-significant (>0.05).

The mean SD of T4 in hypothyroid cases are 11.82 2.98 on comparison with mean SD of controls are 10.97 2.43. P value is statistically non-significant (>0.05).

The mean SD of TSH in hypothyroid cases are 5.48 2.16 on comparison with mean SD of controls are 1.61 0.64. P value is statistically highly significant (<0.001).

Discussion

The study is aimed at evaluating the thyroid profile (T3, T4, and TSH) in hypothyroid and euthyroid pregnant women.

The mean of T3 in hypothyroid cases are 1.32 on comparison with mean of controls are 1.30. P value is statistically non-significant (>0.05).

The mean of T4 in hypothyroid cases are 11.82 on comparison with mean of controls are 10.97. P value is statistically non-significant (>0.05).

The mean of TSH in hypothyroid cases are 5.48 on comparison with mean of controls are 1.61. P value is statistically highly significant (<0.001).(discussion without mentioning SD)

'Long loop" negative feedback mechanism effects hypothalamic pituitary thyroid axis leads to raised levels of TSH in hypothyroidism.[17] The goal of treatment of hypothyroidism in pregnancy is to achieve TSH in first trimester 2.5µIU/ml, in second and third trimester 3 µIU/ml.[18] Depending on the levels of TSH treatment will be given to the patients. Thyroxin replacement will be given in the dose of 50µgr/day, 75µgr/day, and 100µgr/day to the TSH levels of 2.5µIU/ml – 5µIU/ml, 5-8µIU/ml, more than 8µIU/ml.[19] Serum TSH measurements remains the principle determinant of maternal thyroid status at the present time, and should be used to guide treatment decisions and goals.[20]

In the present study the TSH levels in hypothyroid cases are raised which are similar the studies done by Amy J. Blatt et al.[21] Studies from China and India reported a significantly higher TSH reference range for each trimester[22] Trimester specific TSH raised levels are mentioned by the studies done by Yan YQ et al.[23] Vidya et al showed that prevalence of elevated TSH was higher in the high risk group versus the low risk group.[24]

Limitations of the study: TPO and Tg antibodies are not measured as it is cost effective not routinely done at Govt. General Hospital, GUNTUR

Conclusion

Pregnancy is a stress test for thyroid. Thyroid studies are strongly recommended at the time of first obstetrical visit for women diagnosed with thyroid dysfunction before pregnancy. Those are on thyroid replacement therapy and women with risk factors such as family history of thyroid disease must go for the thyroid function test in order to avoid maternal and fetal complication of pregnancy by treatment with levothyroxin.

Conflict of Interest: None.

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